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Filed : June 21, 1999

New claims 43 and 44 relate to the disclosure at page 15 of the description that the subject proteins exhibit antifungal activity. This activity is demonstrated in at least Example 15 at pages 29 and 30. It is also disclosed in the description that the proteins can be utilized as compositions.

The drawings have been replaced primarily in connection with the draftsman's objection thereto. Sheets 4/21, 5/21 and 7/21 to 12/21 have been corrected to properly refer to the particular view comprising a sheet. The drawings are otherwise identical to those of the international application as filed.

The changes made to the claims by the current amendment, including **[deletions]** and **additions**, are shown on an attached sheet entitled **VERSION WITH MARKINGS TO SHOW CHANGES MADE**, which follows the signature page of this Amendment.

Withdrawn Claim

The Examiner has withdrawn claim 34 from consideration as directed to a non-elected invention. The claim has been amended to recite a method of controlling microbial infestation of a plant so that it falls within the scope of the elected invention. Reinstatement of the claim is therefore respectfully requested.

Claim Objection

In connection with the matters raised in item 5 of the Action, the relevant claims have been amended as suggested by the Examiner.

Drawings

The Examiner has objected to the drawings since Figures 13 and 15 are missing portions of Western blots. As a consequence of replacement of the drawings, Figure 13 and 15 now have complete blots shown.

Rejection Under 35 U.S.C. §132

The examiner has objected to the amendment filed September 17, 2001 since she believes that it introduced new matter. However, there is clear support in the specification for this amendment for the reasons that follow.

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Most importantly, it is abundantly clear from the specification as filed that X does not include cysteine. The examiner, in asserting that the September 17, 2001 amendment introduces new matter, points to page 10 of the specification as evidence that there is no support for the amendment. At this very page the following is stated (see lines 6 to 16):

One aspect of the structure that most likely could not be altered without seriously affecting the structure (and, therefore, the activity of the protein) is the content and spacing of the cysteine residues since this would disrupt the formation of disulfide bonds which are critical to a) maintaining the overall structure of the protein and/or b) making the protein more resistant to denaturation and proteolysis (stabilizing the protein structure). In particular, it is essential that cysteine residues reside on one face of the helix in which they are contained. This can best be accomplished by maintaining a three-residue spacing between the cysteine residues within each helix, but, can also be accomplished with a two-residue interval between the cysteine residues – provided the cysteines on the other helical segment are separated by three residues (i.e., C-X-X-C-X-X-X-C-nX-C-X-X-X-C-X-X-X-C where C is cysteine, X is any amino acid, and n is the number of residues forming a turn between the two α -helical segments).

It is clear from the foregoing passage that Applicants intended cysteine to be excluded from the definition of X given at line 15.

Moreover, it is implicit in the language of claim 1 as amended on June 21, 1999 that X cannot be a cysteine residue. The originally amended claim—which was directly extracted from claim 1 of the international application (PCT/AU97/00874)—read as follows:

A protein fragment having antimicrobial activity, wherein said protein fragment is a polypeptide containing a relative cysteine spacing of C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C (SEQ ID Nos: 37-39) wherein X is any amino acid residue, and C is cysteine.

A key recitation is that the polypeptide has a particular spacing of cysteine residues. This spacing is specified in the formula “C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C”. Thus, if any X is a cysteine, the spacing of this residue will not be as specified in the formula.

It is thus a fundamental aspect of the claim as originally drafted that the X of the formula C-2X-C-3X-C-(10-12)X-C-3X-C-3X-C (SEQ ID Nos: 37-39) is any amino acid residue with the exclusion of cysteine. It was therefore not necessary for Applicants to seek to include the qualification “other than cysteine” in claim 1. The amendment is nevertheless sought by Applicants to make it abundantly clear that cysteine is excluded from the definition of X.

In support of the view that the definition of X prior to amendment excludes cysteine, Applicants submit a declaration by Donald J Maclean in which Dr Maclean states at paragraph 6

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that 'in my opinion, therefore, it is implicit within the passage that "X is any amino acid residue" must be read as "X is any amino acid residue other than cysteine"'.

Figure 4 provides further evidence that X is any amino acid residue other than cysteine. This figure is an alignment of various relevant sequences with the alignment based on the C-3X-C motif. If sheet 4/21 is considered, it can be seen that cysteine is not one of the residues that occurs between the two cysteines of a motif or between the two motifs.

In conclusion, the addition of the words "other than cysteine" in no way is an introduction of new matter because this exclusion is consistent with what was disclosed in the application as filed and in any case is implicitly stated in the original claim wording. The amendment does not, therefore, offend against 35 U.S.C. §132.

Rejection under 35 U.S.C. §112, first paragraph and 35 U.S.C. §132

The Examiner has rejected claims 1, 2, 11, 13 and 17-21 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one of skill in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. The basis for this rejection is that the specification provides no support for the amendment to claim 1 that "X is any amino acid residue other than cysteine". To support her position, the Examiner points to disclosures such as at pages 4 and 10 where it is disclosed that "X is any amino acid residue".

The disclosure at page 4, lines 22 and 27 that "X is any amino acid residue" is used in conjunction with the disclosure at lines 21 and 26 of a formula that has a particular spacing of cysteine residues. As discussed above in conjunction with the previous item of the report, for the cysteine spacing to hold, X cannot be a cysteine residue. Consequently, it is implicit in the disclosures at page 4, lines 21 and 26 of polypeptides containing the specified "relative cysteine spacing" that the X residues cannot be cysteine.

With regard to the disclosure at page 10, it was pointed out above in conjunction with the comments on item 7 of the Action that the passage "X is any amino acid residue" must be read in the context of the disclosure as a whole. Earlier at the same page, Applicants state that "[o]ne aspect of structure that most likely could not be altered without seriously affecting the structure...is the content and spacing of the cysteine residues..." (see lines 6 to 8). When "X is

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any amino acid residue" is read together with the earlier statement, it must be read as "X is any amino acid residue other than cysteine".

Further, and again as pointed out in conjunction with item 7 of the Action, the sequence alignment comprising Figure 4 reveals that in all of the sequences included in the alignment, none has a cysteine in the region between the two C-3X-C motifs. Figure 4 thus constitutes actual data that X is any amino acid residue other than cysteine.

In summary, the specification does support the amendment sought to be made in claim 1 and the recitation "X is any amino acid residue other than cysteine" does not constitute the introduction of new matter. Accordingly, Applicants respectfully request that the 35 U.S.C. §112 (first paragraph) rejection be withdrawn.

Rejection under 35 U.S.C. §112, second paragraph

The Examiner has rejected claims 16, 21 and 41-42 under 35 U.S.C. §112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant claims as the invention. The Examiner's basis for this rejection is that claim 16 is indefinite through the recitation of "identifying in a known sequences or designing an amino acid sequence". In particular, the Examiner asserts that claim 16 is unclear because:

- i) the claimed method does not provide any steps as to how to identify in a known sequence [an amino acid sequence which forms a helix-turn-helix structure];
- ii) there is no indication as to whom the sequence is know; and
- iii) it is not apparent which residues would or would not be substituted and in what positions.

Comments on each of these points follow.

Point (i)

Applicants submit that the provision of steps as to how to identify a sequence which forms a helix-turn-helix structure is not necessary. One of ordinary skill in the art would be cognizant of the secondary structure of proteins and the particular amino acids that contribute to secondary structure. Such information can be found in basic biochemistry texts such as *Biochemistry* by Lupert Stryer (Second Edition, W.H. Freeman & Company, San Francisco, 1981). Copies of relevant pages (32-38) of Stryer are **enclosed** for the Examiner's convenience.

One of ordinary skill in the art would also be aware of the publicly available computer programs that can be used to analyze sequences for particular features of secondary structure.

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Such programs are referred to in the instant application (see Example 8 at pages 19 and 20, particularly lines 23 to 34 at page 19).

Armed with knowledge of what constitutes secondary structure, knowledge of the particular amino acid residues that contribute to secondary structure, and having access to analysis programs, the skilled person would readily be able to identify a helix-turn-helix structure in a sequence. The steps are therefore implicit in the recitation and Applicants respectfully submit that it is not necessary to spell them out in the claim.

Point (ii)

The sequence is a sequence known to the skilled addressee. That is, it is a sequence that a skilled person may have established for a protein of interest to that person, or could be a sequence from public information such as a scientific publication or a protein sequence database.

The recitation "known sequence" has been included in the claim to distinguish an existing sequence from a hypothetical sequence that would be the result of "designing a sequence which forms a helix-turn-helix structure". Applicants submit that it would be abundantly clear to a skilled person as to whom the sequence would be known.

Point (iii)

Commenting on this point will be aided by reference to the relevant portion of claim 16 which reads as follows:

- b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 5.

A person of ordinary skill in the art would immediately appreciate on considering the claim that the foregoing step involves comparing the sequence (the test sequence) having a helix-turn-helix structure with SEQ ID NO: 1, SEQ ID NO: 3, or SEQ ID NO: 5. This comparison would allow identification of the residues in the test sequence corresponding to positively charged and cysteine residues in the reference sequences. The skilled person would then immediately know which residues would have to be substituted to give the same distribution of positively charged and cysteine residues in the test sequence as in the reference sequences.

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Contrary to the Examiner's assertion therefore, Applicants believe that the portion of claim 16 rejected by this point is not indefinite.

In view of the foregoing comments on the three points, Applicants submit that withdrawal of the objection is warranted.

The Examiner has maintained her rejection of claim 21 as being indefinite on the basis of inconsistencies between the sequence listings and the claim. However, claim 21 as considered by the Examiner reads as follows:

A homologue of any of the protein fragments of Claim 1.

The rejection is therefore not relevant to claim 21.

In the event that the Examiner intended to reject claim 19 which does have relevant recitations, Applicants point out that this claim was amended in the response filed December 3, 2001 to Paper No. 5.

Rejection under 35 U.S.C. §102(a)

Claim 1 was rejected under 35 U.S.C. §102(a) as being anticipated by Tatar *et al.* (EP 093652, November 9, 1996). The Examiner believed that since Tatar *et al.* discloses peptides to vaccinate against *E. coli* enterotoxins which contain the formula C3XC12XC3XC, that Tatar *et al.* anticipates the peptide of Claim 1. This rejection predicates on the following alignment of a Tatar *et al.* sequence and SEQ ID NO: 39:

| | | | |
|----|---|------------------------|----|
| Qy | 1 | CXXXCXXXXXXXXXXXXXCXXC | 22 |
| | | I:::I:::.....I:::I | |
| Db | 6 | celccnpacagcyntfyccelc | 27 |

The rejection only holds if X of the instant claim can be any amino acid residue. However, if cysteine is excluded as an X residue, it can be seen that the Tatar *et al.* sequence does not conform to the claimed sequence. This is because there is a cysteine between the two cysteines of the C-3X-C motifs and two cysteines between the two motifs—that is, two of the twelve X residues are cysteines.

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Applicants have pointed out above that it was an implicit feature of claim 1 before the amendment to include the "other than cysteine" recitation that cysteine was excluded from the definition of X. Since X cannot be cysteine, the claim does not include the Tatar *et al.* sequence within its scope. Since the Tatar *et al.* sequence is not within the scope of claim 1, the claim is not anticipated by the citation.

The amendment sought to be made to claim 1 to include the recitation that X is any amino acid residue "other than cysteine" merely enforces the already existing novelty of the claim.

Rejection under 35 U.S.C. §102(a)

Claim 1 was rejected under 35 U.S.C. §102(a) as being anticipated by Voerman (WO 96/13585, May 9, 1996). The Examiner believed that since Voerman discloses a medicament and pharmaceutical preparation wherein the sequence comprises SEQ ID NO: 37, it anticipates the claimed invention.

Voerman discloses proteins which include the following two sequences:

CLIFCPNGFAVDENGCELPC; and
CRIYCPKGFEVDENGCELPC.

It can be seen that these sequences include two C-3X-C motifs. However, the motifs are separated by ten residues. Claim 1 as amended recites either eleven or twelve residues between the two C-3X-C motifs. Voerman is not, therefore, an anticipation of claim 1 as presently worded.

Conclusion

Should there be any further questions regarding the above-captioned patent application, the Examiner is respectfully requested to contact the undersigned attorney at the telephone number below.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. 11-1410.

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Respectfully submitted,

KNOBBE, MARTENS, OLSON & BEAR, LLP

Dated: April 4, 2002

By: Jennifer A. Haynes

Jennifer A. Haynes, Ph.D.

Registration No. P48,868

Agent of Record

620 Newport Center Drive

Sixteenth Floor

Newport Beach, CA 92660

JAH-5709.DOC:afa
040202

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend the claims as follows:

1. **(Amended Four Times)** An isolated or purified protein fragment having antimicrobial activity, wherein said protein fragment is a polypeptide comprising a cysteine spacing of C-3X-C-[(10-12)]_nX-C-3X-C (SEQ ID [Nos: 37-] NO: 38 and 39) wherein n is 11 or 12, X is any amino acid other than cysteine, and C is cysteine.

2. **(Thrice Amended)** An isolated or purified protein comprising at least one polypeptide fragment according to claim 1, wherein said polypeptide fragment has a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3 and SEQ ID NO: 5.

16. **(Amended Four Times)** A method of preparing an antimicrobial protein, said method comprising:

- a) identifying in a known sequence or designing an amino acid sequence which forms a helix-turn-helix structure;
- b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;
- c) synthesizing chemically or expressing by recombinant DNA techniques in liquid culture an antimicrobial protein comprising said substituted amino acid sequence; and
- e) isolating said antimicrobial protein.

17. **(Twice Amended)** The protein fragment of [C]claim 1, wherein said protein fragment is a polypeptide containing a relative cysteine and tyrosine or phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOS: 34-36) wherein X is any amino acid residue, and C is cysteine, and Z is tyrosine or phenylalanine.

18. **(Twice Amended)** The protein fragment of [C]claim 1, wherein said relative cysteine spacing comprises C-2X-C-3X-C-(10-12)X-C-3X-C-2X-C (SEQ ID NOS: 1-33) wherein X is any amino acid residue, and C is cysteine.

19. **(Twice Amended)** **[The protein fragment of Claim 1]** An isolated or purified protein fragment having antimicrobial activity, wherein said protein fragment is selected from the group consisting of:

residues 29 to 73 of SEQ ID NO: 1

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residues 74 to 116 of SEQ ID NO: 1
residues 117 to 185 of SEQ ID NO: 1
residues 186 to 248 of SEQ ID NO: 1
residues 29 to 73 of SEQ ID NO: 3
residues 74 to 116 of SEQ ID NO: 3
residues 117 to 185 of SEQ ID NO: 3
residues 186 to 248 of SEQ ID NO: 3
residues 33 to 75 of SEQ ID NO: 5
residues 76 to 144 of SEQ ID NO: 5
residues 145 to 210 of SEQ ID NO: 5
residues 34 to 80 of SEQ ID NO: 7
residues 81 to 140 of SEQ ID NO: 7
residues 33 to 79 of SEQ ID NO: 8
residues 80 to 119 of SEQ ID NO: 8
residues 120 to 161 of SEQ ID NO: 8
residues 32 to 91 of SEQ ID NO: 21
residues 25 to 84 of SEQ ID NO: 22
residues 29 to 94 of SEQ ID NO: 24; and
residues 31 to 85 of SEQ ID NO: 25.

20. **(Twice Amended)** The protein fragment of [C]claim 1 which is truncated, wherein said truncated protein fragment retains the antimicrobial activity of the nontruncated protein fragment.

21. **(Amended)** A homologue of any of the protein fragments of [C]claim 1.

30. **(Amended)** A composition comprising **[an antimicrobial protein]** a protein fragment according to claim [22] 19 together with an agriculturally-acceptable carrier diluent or excipient.

31. **(Amended)** A composition comprising **[an antimicrobial protein]** a protein fragment according to claim [22] 19 together with a pharmaceutically-acceptable carrier diluent or excipient.

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34. (Twice Amended) A method of ~~inhibiting~~ controlling microbial infestation of a plant, the method comprising treating said plant with an effective amount of the composition according to claim 11 for a period sufficient to inhibit microbial infestation of the plant.

39. (Amended) A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim ~~[30]~~ 31.

42. (Amended) The method of ~~[C]~~claim 16, further comprising testing the antimicrobial protein for antimicrobial activity.